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MINIREVIEW

...le CD95 (APO-1/Fas) and the TRAIL (APO-2L) Apoptosis Systems

Henning Walczak and Peter H. Krammer[1]

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THE CD95 APOPTOSIS SYSTEM

In 1989 we published a paper describing the discovery of an apoptosis-inducing receptor [1]. We named this receptor APO-1, anticipating that it might be the first of a series of apoptosis-inducing receptors. Yonehara *et al.* [2] also described a cell surface molecule named Fas that could be triggered to induce cytotoxicity by an agonistic monoclonal antibody. Sequencing and cloning of the APO-1/Fas proteins and cDNAs, respectively, showed that APO-1 and Fas were identical [3, 4]. The 5th Workshop on Leukocyte Typing [5] suggested the name CD95 for the receptor. CD95 (APO-1/Fas) belongs to the subfamily of death receptors which is part of the TNF-receptor (TNF-R) superfamily. Members of this family are characterized by two to five copies of cysteine-rich extracellular repeats. Death receptors have an intracellular death domain (DD). The DD is essential for transduction of the apoptotic signal.

CD95 is a widely expressed glycosylated cell surface molecule of approximately 45 to 52 kDa (335 amino acids). It is a type I transmembrane receptor and can also occur in several soluble forms [3, 4, 6]. Soluble CD95 is generated by differential splicing with the transmembrane part spliced out. The human CD95 gene, APT, was localized to chromosome 10q23 and the mouse gene to chromosome 19 [7, 8]. Expression of the CD95 gene and cell surface protein are enhanced by IFN-γ and TNF and by activation of lymphocytes [9–11]. Under physiological conditions CD95-mediated apoptosis is triggered by the natural ligand (L) of the receptor, CD95L. CD95L is expressed in a more restricted way than CD95. CD95L was cloned from the cDNA of a killer cell (PC60-d10S) and shown to be a TNF-related type II transmembrane molecule [12]. The mouse and human CD95L genes were mapped to chromosome 1 [13, 14]. Killer cells expressing CD95L were shown to kill target cells in a Ca^{2+}-independent fashion via CD95–CD95L interaction [15]. In addition, human CD95L overexpressed in COS cells was found in the supernatant and induced apoptosis in a soluble form [16–19]. Soluble CD95L is found as a trimer and is generated from the transmembrane form by the activity of a metalloprotease [20–22]. Several papers report activity of the soluble ligand, whereas other papers show the contrary depending on the target cells used [23].

ROLE OF THE CD95/CD95L SYSTEM IN DELETION OF PERIPHERAL T CELLS AND IN LIVER HOMEOSTASIS

Results by us and those by others *in vitro* and *in vivo* suggest that CD95 and its ligand, CD95L, are involved in TCR-triggered apoptosis. T cell apoptosis occurs as "fratricide" by interaction of the membrane-bound receptor with the membrane-bound ligand on neighboring T cells that kill each other. TCR-triggered CD95-mediated apoptosis is also found in single Jurkat T cells. A single TCR-activated T cell in the absence of costimulation may autonomously decide to die by apoptosis employing, at least in part, the CD95 pathway. These results suggest a minimal model in which TCR-induced death in activated T cells involves CD95/CD95L-mediated suicide. Collectively, TCR-induced CD95-mediated apoptosis may occur in several forms: fratricide, paracrine death, and autocrine suicide [24]. The TCR/CD95/TNF-R death mechanism has shed new light on peripheral T cell tolerance by deletion, on suppression of the immune response, and on development of memory in the surviving T lymphocyte pool. In addition, the CD95/CD95L system has an essential role for B cell deletion similar to the one described for T cells. Here, however, B cell deletion might be brought about by CD95L-positive T cells which kill CD95-positive B cells [25].

The CD95/CD95L system also plays an important role in another organ: the liver. CD95 is expressed in the developing [26] and the mature [11] liver. Primary hepatocytes from mice [27] and man [28] were demonstrated to be sensitive towards CD95-mediated apoptosis *in vitro*. A physiological role of CD95 in maintaining liver homeostasis has been suggested since mice defi-

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cient in CD95 develop increased cellularity and substantial liver hyperplasia [29].

THE BIOCHEMICAL MECHANISM OF CD95-INDUCED APOPTOSIS

Oligomerization of CD95 is a prerequisite for the transduction of the apoptotic signal. CD95 dimers do not induce apoptosis [30]. The most probable structure to transmit an apoptotic signal is a CD95 trimer, as this structure corresponds to the predicted trimeric structure of members of the TNF-R superfamily by X-ray crystallography (e.g., LTα complexed with TNF-R1 [31]). When CD95 was discovered the use of a known signaling pathway could not be deduced or predicted from the sequence of the intracellular part of the receptor. A deletion of 15 amino acids of the C-terminus of CD95 was shown to increase CD95-mediated apoptosis. Further deletions inhibited the CD95 signal completely. When the sequence of the intracellular part of CD95 was compared with the one of TNF-R1 a homology region of 68 amino acids could be defined.

Moreover, using deletion and point mutagenesis, Tartaglia *et al.* [32] defined a region of TNF-R1 that was essential for the cytotoxicity mediated by the receptor. This stretch was 80 amino acids long and comprised the domain described by Itoh and Nagata [33], the "death domain" (DD). The DD also contains the valine residue (Val_{238}) mutated in lpr^{cg} mice that abolishes apoptosis signaling. Several years later additional DD-containing receptors were isolated (TRAMP, TRAIL-R1, TRAIL-R2, and DR6). Also, further intracellular DD-containing proteins were found, two of which bind to CD95: FADD/Mort1 [34, 35] and RIP [36]. These molecules as other CD95 binding molecules were cloned by the yeast two hybrid system with the cytoplasmic part of CD95 used as a bait. The yeast two hybrid system selects for low affinity interactions. Therefore, some of these molecules still have to prove their importance *in vivo*. The two DD-proteins FADD and RIP bind to the DD of CD95 directly. Overexpression of FADD and RIP causes apoptosis.

Except for the DD at the C-terminus no other known protein domain could initially be identified for FADD/Mort1. Interestingly, expression of the DD of FADD/Mort1 alone did not cause suicide of the cells, whereas expression of the N-terminus did. This is the reason why this part of the protein is called the "death effector domain" (DED).

In the yeast two hybrid system RIP showed affinity to CD95 and to a lesser degree to TNF-R1. RIP contains a domain homologous to the protein kinases and its DD alone is sufficient to kill a cell.

A negative regulatory role has been suggested for the C-terminus of CD95 because the deletion of the last 15 amino acids of CD95 increases the sensitivity towards CD95-mediated apoptosis [33]. This region of CD95 was claimed to interact with FAP-1 (for "Fas-associated-phosphatase 1"), a protein phosphatase supposedly capable of inhibiting CD95-mediated apoptosis. Recent reports, however, could not show the interaction of FAP-1 with CD95 in mice [37]. In addition, deletion of the 15 carboxyl-terminal amino acids of mouse CD95 did not show any effect on CD95-mediated apoptosis.

Several other partly classical signaling pathways were described and thought to play a role in generating the CD95 death signal. Ceramide is cleaved from membrane sphingolipids by sphingomyelinases (SMases). Sphingolipids form a central part of the cell membrane. Several authors have reported that acid SMase [38, 39] and neutral SMase [40] are essential for CD95-mediated apoptosis. In addition, tyrosine phosphorylation was described as a regulatory modification of many receptors. Thus, protein tyrosine kinases (PTK) were also found essential for CD95-mediated apoptosis [41]. Furthermore, protein kinase C (PKC) may play a role in modulating the cytotoxic signal. Thus, PKC inhibitors such as Bisindolylmaleimide VIII, H7, and HA 1004 sensitized cells toward CD95-mediated apoptosis, whereas activation with the phorbol ester PMA led to resistance [42, 43].

The three-dimensional structure of the CD95 DD has been determined by nuclear magnetic resonance spectroscopy. It consists of six antiparallel, amphipathic α helices arranged in a novel fold, which is likely to be important for binding intracellular signaling molecules [44].

A complex of proteins was identified that associated only with stimulated CD95 [45]. Treatment of CD95-positive cells with the agonistic mAb anti-APO-1 and subsequent immunoprecipitation of CD95 with protein A–Sepharose resulted in the identification of four cytotoxicity-dependent *A*PO-1-associated *p*roteins (CAP1-4). The CAP1-4 proteins could be revealed on 2D-IEF/SDS gels within seconds after CD95 triggering. Together with CD95 these proteins formed a complex called the death-inducing signaling complex (DISC). CAP1 and CAP2 could be identified as two different serine-phosphorylated species of FADD/Mort1. While the binding of FADD/Mort1 to CD95 is stimulation dependent the phosphorylation of FADD/Mort1 is not. Therefore, the role of FADD/Mort1 phosphorylation presently remains a mystery. It is not excluded, however, that it is important to give the entire DISC a structural conformation in order to function properly.

When FADD-DN (the C-terminal DD-containing part) was stably transfected into cells the DISC also formed. However, FADD-DN was recruited to CD95 instead of the endogenous FADD. Analysis on two-dimensional gels revealed that CAP3 and CAP4 were not part of the DISC anymore [46]. These proteins were therefore prime candidates for the signaling molecules. Using nanoelectrospray tandem mass spectrometry se-

quence information of CAP4 was obtained that led to the retrieval of a full-length cDNA clone encoding all sequenced CAP4 peptides [47]. CAP4 contained two DED at its N-terminus and showed the typical domain structure of an ICE-like protease at its C-terminus. It was therefore termed FLICE (for FADD-like ICE). The same molecule was also found by two other groups and was named MACH and Mch5 [48, 49]. FLICE belongs to a family of cysteine proteases, now called caspases [50]. FLICE has received the name caspase-8. Thus, identification of caspase-8 and its location in the DISC upon CD95 triggering connected two levels in the CD95 apoptosis pathway, the CD95 receptor level with the intracellular level of the apoptosis executioner, the caspases.

The finding that caspase-8 was identified as part of the *in vivo* CD95 DISC suggested that its activation occurred at the DISC level. It has indeed been shown that the entire cytoplasmic caspase-8 is converted into active caspase-8 subunits at the DISC [51]. After stimulation FADD/Mort1 and caspase-8 are recruited to CD95 within seconds after receptor engagement. Direct binding of caspase-8 may cause structural changes in the molecule that result in autoproteolytic activation. The active subunits p10 and p18 are released into the cytoplasm. Part of the caspase-8 prodomain stays bound to the DISC. Anti-caspase-8 monoclonal antibodies show that from the eight published caspase-8 isoforms only two, caspase-8/a and 8/b, were expressed predominantly on the protein level in 13 different cell lines tested [52]. Both isoforms are recruited to the DISC and are processed with similar kinetics. Recombinant caspase-8 lacking the prodomain was recently reported to cleave caspase-8 *in vitro*. This suggested autocatalytic cleavage of caspase-8 at the DISC and an amplification step with caspase-8 at the top of a caspase cascade [53, 54]. However, Medema *et al.* [51] used isolated DISC and could not confirm this observation. Recombinant caspase-8 lacking the prodomain might therefore display a different substrate specificity compared to full-length caspase-8 *in vivo*. Most investigators believe that active caspase-8 cleaves various cellular death substrates, including other caspases such as the executioner caspase-3, thus initiating the execution of apoptosis. Furthermore, caspase-8 has been shown to be cleaved and activated by granzyme B in perforin killing by cytotoxic T lymphocytes. Thus, caspase-8 is the primary, though not the only, initiator of a protease cascade also in this type of T killer cell activity [55].

TWO DIFFERENT CD95 SIGNALING PATHWAYS IN TYPE I AND TYPE II CELLS

Regarding the CD95 signaling pathway several controversies are apparent concerning DISC formation, ceramide involvement, Bcl-2 inhibition and DD associating molecules. Most of these controversies have not yet been resolved, but data by Scaffidi *et al.* [56] might provide more clarity to the picture.

Two cell types that each preferably use one of two different CD95 signaling pathways have been identified. These pathways are initiated regardless of whether CD95 triggering is induced by agonistic antibodies or by trimerizing CD95L [57]. In both, type I and type II cells, mitochondria were activated equally upon CD95 triggering, and all mitochondrial apoptogenic activities were blocked by high-level Bcl-2 overexpression. In type I cells, the induction of apoptosis was accompanied by the activation of large amounts of caspase-8 by the DISC, followed by the rapid cleavage of caspase-3 prior to loss of mitochondrial transmembrane potential ($\Delta\Psi_m$). In contrast, in type II cells DISC formation was strongly reduced and activation of caspase-8 and of caspase-3 occurred following the loss of $\Delta\Psi_m$. In type II but not in type I cells high-level Bcl-2 overexpression blocked caspase-8 and caspase-3 activation and apoptosis. CD95-mediated apoptosis in type II cells and in type I cells is dependent and independent of mitochondrial activity, respectively. It is still not completely clear how mitochondria are activated in both type I and type II cells. Yet, the BH3 domain-containing Bcl-2 family member Bid, cleaved by caspase-8 into truncated Bid, has been suggested to fulfill this function [58, 59]. Nevertheless, the mitochondria are only used in type II cells to initiate the executionary apoptosis caspase cascade. Thus, these data clearly establish that CD95-mediated apoptosis uses two pathways probably dependent on the quantity of caspase-8 initially activated.

Recently, this type I–type II concept and the distinction of the two different apoptotic pathways by inhibitory effects of Bcl-2/Bcl-x_L on the type II, but not on the type I pathway, has been challenged [60]. Huang *et al.* argue that while agonistic CD95 antibodies allowed for a distinction of type I and type II cells this difference is undetectable upon apoptosis triggering with CD95L, independent of whether the ligand was crosslinked or not. Instead of immunoprecipitating the CD95 DISC, however, the authors studied whether FADD/Mort1 and Caspase-8 were associated with the plasma membrane. Thus, it remains unclear whether these proteins are indeed recruited to the CD95 receptor or whether they are in membrane-associated aggregates that cannot be dissociated from the plasma membrane after apoptosis induction. The other notion brought forward by Huang *et al.* is that at certain levels overexpression of Bcl-2 or Bcl-x_L does not inhibit CD95L-induced apoptosis while cytotoxic drug-induced apoptosis was efficiently blocked. We have obtained similar data with LZ-CD95L and anti-APO-1 antibodies on both, Jurkat cells transfected with either Bcl-2 or Bcl-x_L and CEM

cells transfected with Bcl-x_L, when the expression of the respective Bcl-2 family members was not kept at high levels. Yet, at high level Bcl-2 or Bcl-x_L expression the difference was still as apparent with the CD95L as it was with anti-APO-1 antibodies. Therefore, the data by Huang *et al.* [60] do not challenge the type I–type II concept.

Nevertheless, the reason for the observed difference in caspase-8 activation by the CD95 DISC in type I versus type II cells remains undefined. Hypothetically, biochemical differences at the CD95 receptor itself might provide the answer.

FLIPs (FLICE INHIBITORY PROTEINS)

Other DED containing proteins have been found in the data bases. Some of these proteins are encoded by class γ herpes viruses such as herpes virus Saimiri (HVS), by human herpes virus 8 (HHV 8), a Karposi sarcoma-associated herpes virus, and by moluscum contagiosum [61]. The proteins have been called v-FLIPs (for viral FLICE inhibitory proteins). v-FLIPs consist of two DEDs and biochemical analysis of v-FLIP-transfected cells showed that they bind to the CD95/FADD complex and thus inhibit the recruitment of caspase-8 and a functional DISC formation. In transfected cells, v-FLIP was capable of inhibiting apoptosis induced by several apoptosis-inducing receptors (CD95, TNF-R1, TRAMP/DR3, and TRAIL-R1). This suggests that these receptors use similar signaling pathways [62–64]. A human homologue of v-FLIP has been identified independently by several groups and has thus received a plethora of different names: c-FLIP/FLAME/I-FLICE/Casper/CASH/usurpin/MRIT/CLARP [63, 65–71]. c-FLIPs occur in two forms, a short form and long form. The short form seems to act like v-FLIP. The long form has a sequence similar to caspase-8 but has an inactive enzymatic site and interferes with the generation of active caspase-8 (FLICE) subunits at the receptor level. The gene of c-FLIP/usurpin has been identified. It is composed of 13 exons and is clustered within approximately 200 kB with the caspase-8 and -10 genes on human chromosome 2q33 to 34 [69, 72]. Taken together, the function of the c-FLIPs is not entirely clear, as data vary and reach from inhibition to induction of apoptosis.

THE TRAIL (APO-2L) APOPTOSIS SYSTEM

In 1995 the TNF-related apoptosis-inducing ligand, TRAIL, was identified purely on the basis of sequence homology to the other members of the TNF family [73]. The revealing homology resided within two short but highly conserved sequence motifs characteristic for TNF family members. Among the members of this family CD95L shares highest sequence homology with TRAIL. In addition, TRAIL was capable of inducing apoptosis. Interestingly, however, TRAIL induced apoptosis in many of the tumor cell lines that were tested yet normal cells were not killed.

In order to understand the function and regulation of the TRAIL apoptosis-inducing system the TRAIL receptor had to be identified. The outcome of the resulting cloning race was surprising. With many different receptors at its disposal TRAIL emerged as the most promiscuous of all cytokines known (reviewed in [74]). TRAIL can bind two apoptosis-inducing receptors, TRAIL-R1 (DR4) and TRAIL-R2 (Killer, DR5, TRICK2), two additional cell-bound receptors incapable of transmitting an apoptotic signal, TRAIL-R3 (LIT, DcR1) and TRAIL-R4 (TRUNDD, DcR2), and, last, a soluble receptor called osteoprotegerin (OPG). OPG does not only bind to TRAIL but also to another member of the TNF family, the osteoclast differentiation factor (ODF, OPGL, RANKL) [75]. At first it seemed as if the existence of these functionally distinct receptors might provide an answer to the differential sensitivity to TRAIL observed between normal and transformed cells. The initial findings indicated that TRAIL-R3 and TRAIL-R4 may act as so-called "decoy receptors" by competing with TRAIL-R1 and TRAIL-R2 for binding of TRAIL [76, 77]. However, these results were obtained solely by relying on overexpression data. By using monoclonal antibodies specific for each one of the individual surface-bound TRAIL receptors it was recently shown for various cellular systems that TRAIL resistance is controlled intracellularly rather than at the level of TRAIL-R3 and/or TRAIL–R4, the putative "decoy receptors". In melanoma cells TRAIL-R expression levels did not correlate with sensitivity towards TRAIL [78–80]. In addition, in primary versus transformed keratinocytes the levels of cFLIP inversely correlated with sensitivity to TRAIL and TRAIL-R1/R2-specific antibodies whereas TRAIL-R3 and -R4 were not involved [81]. When human blood-derived dendritic cells (DC) were examined we found that immature DC were susceptible to TRAIL, whereas mature DC were not. Again, while the TRAIL-R surface expression pattern did not change upon DC maturation c-FLIP levels increased over time in DC. The increase in cFLIP expression may thus be responsible for TRAIL resistance of fully mature DC [82]. Regarding T cells, we have not been able to kill significant amounts (always less than 5%) of human peripheral T cells with TRAIL regardless of their activation status [83]. Yet, upon incubation with cycloheximide T cells become TRAIL sensitive and, again, sensitization of the T cells is not regulated at the receptor level but rather due to the disappearance of intracellular resistance factors [83]. Thus, the "decoy" concept awaits confirmation in nonoverexpression systems and the biochemical function of the nonapoptosis-

inducing receptors, TRAIL-R3, TRAIL-R4, and OPG, remains unsolved. Taken together, c-FLIP seems to be one of the prime candidates as an intracellular regulator of TRAIL sensitivity versus resistance in a number of different cell types. Yet, other intracellular inhibitors of apoptosis are likely to also be involved in resistance to TRAIL [80].

BIOCHEMICAL PATHWAY OF TRAIL-INDUCED APOPTOSIS

Soon after its discovery TRAIL was shown to use a caspase-dependent pathway to kill its target cells [84]. In order to understand the signaling pathways triggered by TRAIL stimulation in more detail we now studied the exact sequence of intracellular biochemical events following TRAIL stimulation. TRAIL-induced apoptosis involves late dissipation of the mitochondrial membrane potential and cytochrome c release that follows activation of caspase-8 and caspase-3 and induction of DNA fragmentation. In addition, inhibition of caspase-8, but not caspase-9 or -3, prevents mitochondrial permeability transition (PT) and apoptosis. Various cell lines overexpressing the antiapoptotic proteins Bcl-2 or Bcl-x_L are not or only marginally protected against TRAIL-induced apoptosis. In contrast, apoptosis induced by the chemotherapeutic drug etoposide was severely impaired in these cells. Thus, TRAIL induces apoptosis via a caspase signaling cascade that executes apoptosis independently of the pro-apoptotic machinery of mitochondria [85].

The question remained which molecules actually transmit the TRAIL death signal under native conditions. When the TRAIL-Rs were cloned this question was addressed in a number of studies. Again, they solely relied on overexpression of possible interaction partners. Thereby, a tremendous number of proteins known to be involved in various aspects of apoptosis signaling have been suggested to also play a role in TRAIL-induced apoptosis (reviewed in [86]). In particular a discussion arose concerning the use of FADD in TRAIL-induced apoptosis. In order to solve this question we directly analyzed the TRAIL DISC under native conditions in analogy to our studies of the CD95 DISC. This analysis revealed that both FADD/Mort1 and caspase-8 are recruited to the TRAIL DISC in all cell lines tested (BJAB, BL60, and Jurkat) and that these two proteins constitute integral components of the TRAIL-R2-DISC [87]. They are in fact necessary for the TRAIL-R2 DISC to be functional as Jurkat cells that lack either FADD/Mort1 or caspase-8 are completely resistant to apoptosis induced either by TRAIL-R2-specific agonistic antibodies or by TRAIL itself [87]. It remains to be solved whether TRAIL-R1- or heteromeric TRAIL-R1/R2-DISCs also use these signalling molecules or whether the various possible complexes use different pathways to signal cell death.

PHYSIOLOGICAL ROLE OF THE TRAIL APOPTOSIS SYSTEM

For quite some time after the identification of TRAIL and its receptors the physiological function of this novel apoptosis-inducing system remained unknown. Recently, in a number of studies the functional expression of TRAIL was discovered on the surface of different cells that had previously been known to induce apoptosis in target cells by an unknown mechanism. Among them are type II interferon (IFN-γ)-stimulated monocytes [88], cytomegalovirus (CMV)-infected fibroblasts [89], type I IFN- (IFN-α and IFN-β), or TCR-stimulated T cells [90–92], nonstimulated $CD4^-$ T cells [78, 93–95], IFN-α- and IFN-γ-stimulated, as well measles virus-infected DC [96, 97] and natural killer (NK) cells [91, 98–100]. Interestingly, functional surface expression of TRAIL was often associated with stimulation by interferons. Therefore, it is likely that the antitumoral effect of IFNs may at least be partially mediated by TRAIL-induced direct killing of TRAIL-sensitive tumor cells [88, 92]. Yet, the main physiological function attributed to IFNs resides in their antiviral activity. In fact, Sedger *et al.* showed that TRAIL-induced apoptosis specifically in virally infected cells while sparing noninfected normal cells [89]. Thus, the TRAIL system might have evolved in order to control viral transformation. Thus, the fact that many oncogenically transformed cells are also sensitive to TRAIL might be a beneficial side effect of the function of TRAIL to kill virally transformed cells. In any case, further insight into the physiological function of TRAIL will be provided by the analysis of mice that are deficient for this cytokine.

Given the different TRAIL receptors it will be a complex undertaking to determine which individual receptor is responsible for the different physiological functions exerted by TRAIL. As mentioned before, the physiological function of the nonapoptosis-inducing receptors for TRAIL remains undefined. On the other hand the apoptosis-inducing receptors for TRAIL have to be involved on the target side of the newly discovered physiological activities of TRAIL. However, it is still unclear which one of the two receptors or combination thereof serves as the transducer of the apoptotic signal in different physiological and pathophysiological situations. Studies with monoclonal antibodies capable of blocking or inducing individual receptors will provide useful information. In addition, experiments with mice deficient for one or a combination of the TRAIL receptors will prove to be crucial in the determination of the function of each individual receptor. At this point, however, only one mouse TRAIL receptor has

been identified and this receptor is capable of inducing apoptosis [101]. It will be interesting to see whether all human TRAIL-R will have a murine counterpart.

ANTI-TUMOR POTENTIAL OF TRAIL

The other known apoptosis-inducing members of the TNF family, CD95L and TNF, are detrimental upon systemic administration. The property of TRAIL to kill tumor cells more efficiently than normal cells prompted us to test the antitumor potential of TRAIL *in vivo*. We showed that TRAIL was capable of inhibiting tumor growth in mice in the absence of any overt toxicity [102]. Another group that had independently discovered TRAIL and had named it APO-2L [103] then showed that the *in vivo* effect of TRAIL/APO-2L could also be observed in cynomolgous monkeys and that TRAIL could act in synergy with chemotherapeutic drugs [104].

A number of studies have been performed combining TRAIL with different chemotherapeutic drugs (reviewed in [105]). Most chemotherapeutic drugs and radiation therapy used in the treatment of malignancies lead to apoptosis primarily by engagement of the mitochondrial proapoptotic machinery. Upon treatment tumors often acquire resistance to conventional antitumor therapy. In many cases Bcl-2 or Bcl-x_L overexpression is the main reason for chemotherapy resistance. As mentioned above, TRAIL can bypass the antiapoptotic effect of Bcl-2 or Bcl-x_L overexpression [85]. These data suggest a novel alternative antitumor strategy by using TRAIL against Bcl-2- or Bcl-x_L-overexpressing tumors. Preferably, though, one would envisage a treatment that combines the cytotoxic potential of TRAIL with that of chemo- and/or radiotherapy. By attacking the tumor cells from two different angles such a combinatorial treatment of cancer will most likely diminish the chances of the tumor to develop a therapy-resistant variant. This is due to the fact that TRAIL and chemotherapeutic agents work primarily via distinct apoptotic pathways. While TRAIL preferentially makes use of the direct caspase pathway that bypasses mitochondria most chemotherapeutic agents and radiation exert their apoptotic potential primarily via the mitochondrial apoptotic pathway.

Thus, the treatment of cancer by direct induction of apoptosis in tumor cells, first proposed upon identification of the anti-APO-1 antibody [1], may now become reality as clinical trials with TRAIL/APO-2L are bound to start soon.

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